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SYNTHESIS, DOCKING STUDIES AND ANTIOXIDANT ACTIVITY OF TETRAPEPTIDE FGVY

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ABSTRACT
A rational designing of linear Tetrapeptide FGVY was done and was synthesized by solution phase peptide synthesis. The docking studies of designed linear tetrapeptide FGVY was carried out by using Schrodinger Software Solutions, USA. Qikprop results show the ligand FGVY mostly act as antihypertensive and anti coagulant properties. The solution phase synthesis of FGVY is carried out by using Dicyclohexyl carbodiimide (DCC) as coupling agents and triethyl amine as base. Structure of synthesized FGVY was confirmed by FTIR, ^{1}H NMR and Mass spectral data, and evaluated for antioxidant property by using 1,1-diphenyl-2-picryl-hydrazil (DPPH$^{\cdot}$) method and the synthesized peptides FGVY possess moderate antioxidant activity.
KEYWORDS: Tetrapeptide FGVR, Solution Phase Peptide Synthesis, Schrodinger software 2009 (Docking), Antioxidant activity.

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INTRODUCTION

Reactive oxygen species (ROS), capable of causing damage to DNA, has been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age[1,2]. ROS are continuously produced during normal physiologic events and they can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. However, they are removed by antioxidant defence mechanisms. The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate and tert-butylhydroquinone. However, BHA and BHT have suspected of being responsible for liver damage and carcinogenesis[3]. Therefore, there is a continuous interest for new antioxidants. In low concentrations, synthetic antioxidants are also in use for many industrial processes e.g. inhibition of radical formation for preventing premature polymerization during processing, storage and transportation of unsaturated monomers. They exert their effects by scavenging or preventing the generation of ROS[4] which can protect the formation of free radicals and retard the progress of many chronic diseases[5] including cancer, neurodegenerative, inflammation and cardiovascular diseases[6].

Docking is frequently used to predict the binding orientation of small drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

Peptides are the important class of organic compounds with potent biological activities. Peptides function as hormones, enzymes, enzyme inhibitors or substrates, growth promoters or inhibitors, neurotransmitters and immuno modulators. Most of the peptides exhibit their biological activities[7-10] through binding to corresponding acceptor molecules (receptors or enzymes). In the present work the designed ligand FGVY (Phe-Gly-Val-Tyr) was targeted to the cancer cell proteins, human peptide deformylase protein and HIF 1α protein using Schrodinger software and was synthesized by solution phase peptide synthesis.

MATERIALS AND METHODS

Computer Aided Designing of Anticancer Peptides - Molecular docking: In the present work Schrodinger 2009 software was used to dock the ligand with the target protein. The designed ligand FGVY tetrapeptide docked against target protein Human Mitochondrial peptide deformylase and hypoxia-inducible factors HIF-1α.In standard virtual docking studies, ligand is docked into the binding site of a receptor where the receptor is held rigid and the ligand is free to move. Molecular docking involves the following steps (Schrodinger Software Solutions, USA), 1) Ligprep, 2) Protein preparation wizard, 3) Glide grid generation, 4) Docking.

Ligprep: Ligprep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SD or Maestro format. The resulting structures can be saved in either SD or Maestro format.

Protein preparation wizard: The typical structure file from the PDB is not suitable for immediate use in molecular modeling calculations. A typical PDB structure file consists only of heavy atoms and may include a co crystallized ligand, water molecules, metal ions, and cofactors. Some structures are multimeric, and may need to be reduced to a single unit. Because of the limited resolution of X-ray experiments, it can be difficult to distinguish between NH and O, and the placement of these groups must be checked. PDB structures may be missing information on connectivity, which must be assigned, along with bond orders and formal charges. Schrodinger has therefore assembled a set of tools to prepare proteins in a form that is suitable for modeling calculations.

Glide grid generation: Glide searches a favorable interaction between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide more accurate scoring of the ligand poses.

Docking: Protein-Ligand docking is a molecular modeling technique is used to predict the orientation and confirmation of binding of ligands with proteins (Fig 1). The increase in negative value of glide score more is the interaction between the ligand and target protein Table 1. The preliminary study of docking shows that the designed ligand tetrapeptides docks with the target proteins, Human Mitochondrial peptide deformylase protein and HIF-1α protein. This shows that the ligands tetrapeptide can bind effectively to the predicted protein. This is supported with the experimental procedure by wet lab method.

Analytical grade solvents and commercially available reagents were used without further purification. Anhydrous conditions for all the reactions were conducted in dried apparatus. All the reactions were magnetically stirred unless otherwise stated. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by capillary method. Amino acids, di-tert-butylpyrocarbonate, trifluoroaceticacid, DCC, Diethyl ether, Methanol and Chloroform were obtained from and Spectrochem Ltd, Mumbai. DPPH was obtained from AVRA. IR spectra were recorded on FTIR spectrometer using a thin film support on KBr pellets. The values are reported as ν_{max} (cm^{-1}). ^1H NMR spectra was recorded on ^1H NMR Brucker JOEL (400MHz) NMR spectrometer. The spectra was obtained in $CDCl_3$ and the chemical shift values are reported as values in ppm relative to TMS (δ=0) as internal standard. FAB Mass spectra were recorded. In order to carry out the synthesis the dipeptides Boc-Phe-Gly-OMe and Boc-Val-Tyr-OMe were properly appropriated and coupled together to get the linear tetrapeptide (Scheme 1).

Preparation of Dipeptides: Amino acid methyl ester HCl (10 mmol) was dissolved in chloroform ($CHCl_3$) (20 mmol). To this triethylamine (Et_3n) (4 ml, 28.7 mmol) was added at 0^0C and the reaction mixture was stirred for 15 minutes. Boc amino acid (10 mmol) in chloroform (20 ml) and DCC (Dicyclohexyl Carbodiimide) (2.2gm, 10mmol) were added with stirring. After 16hrs, the reaction was filtered and the residue was wished with $CHCl_3$ (30ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20 ml) and plain water (20 ml).The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4), filtered and evaporated in vacuum. To remove the traces of Dicyclohexyl urea (DCU), the product was dissolved in minimum amount of $CHCl_3$ and cooled to 0^0C.The crystallized DCU was removed by filtration. Petroleum ether was added to the filtrate at 0^0C to recrystallize the pure product. Boc-Phe-Gly-OMe and Boc-Val-Tyr-OMe were prepared in this manner.

Preparation of linear Tetrapeptide: The ester group of the dipepeptide (Boc-Phe-Gly-OMe) was removed and the Boc-group of another dipeptide (Boc-Val-Tyr-OMe) was deprotected. Both the deprotected units were coupled to get the linear tetrapeptide.

Antioxidant activity

Synthesized linear tetrapeptide FGVY screened for antioxidant activity such as free radical scavenging activity. The free radical scavenging activity of the synthesized compounds was determined by the 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$).This activity was measured by following the method described by Ilhami

Gülçin et al[11], where in the bleaching rate of a stable free radical, DPPH· is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 1 mL of 0.1 mM methanolic solution of DPPH was added to 3 mL of the synthesized samples FGVY, at different concentrations in methanol (10, 20, 50, 100 µg/mL). The samples were kept in the dark for 30 min after which the absorbance was measured at 517 nm in a UV spectrophotometer (Systronics 2202). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Butylated Hydroxy Toluene (BHT), which is a good antioxidant, is taken as a standard in this study. The linear tetrapeptide FGVY showed moderate free radical scavenging activity at all different four concentrations studied. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH· Scavenging effect (%) = $[(A_c-A_s)/A_o)\ 100]$

Where A_c is the absorbance of the control reaction and A_s is the absorbance in the presence of sample.

RESULTS AND DISCUSSION

Docking: A Preliminary study was initially carried out with Schrodinger software where the Designed ligand FGVY was docked with Human Mitochondrial peptide deformylase and HIF 1α that were collected from PIR (Protein information resource) (listed in Table 1) and their docking score in Tabke:2. The docking score revealed that the designed ligand FGVY was able to bind to the protein HIF-1α effectively.

Synthesis: A rational designing of linear Tetrapeptide was done and were synthesized by solution phase peptide synthesis[12]. The results of all the peptides along with their physical properties have been shown in Table 2. The final synthesized compound was obtained in a good yield and is shown below:

Spectral Analysis: The structure of the synthesized compound was characterized by FT-IR, ^{1}H NMR and FAB-MS. ^{1}H NMR spectrum (δ, ppm): 7.0-7.2 (5H, m, Ar-H), 6.8-7.0 (4H, d, Ar-H), 6.4-6.5 (1H, S, OH), 4.2-5.2 (5H, S, αH-H), 3.6-3.7(3H, m, OCH_3), 3.1-3.5 (4H,S, NH), 0.8-2.3 (20H, br, Bz-H, βH, γH, of Phe, Tyr, Val and Boc), IR spectrum (v/cm^{-1}): 3433.2 cm^{-1} (OH stretch), 3287.38 cm^{-1} (NH stretch), 3017 cm^{-1} (Ar-CH stretch), 2935 cm^{-1} (Alip-CH stretch) 1652 cm^{-1} (C=O stretch. The molecular ion peak was obtained at 587 (M+2).

Antioxidant activity: The result of sample was compared with the standard (butyl hydroxyl toluene-BHT). With this method it was possible to determine the antiradical power of an antioxidant compound by measuring the decrease in the absorbance of DPPH at 517 nm. A color change from purple to yellow indicated that the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form stable DPPH molecule. Table 3, illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of prepared sample and standards.

CONCLUSION

In conclusion, we have synthesized a linear tetrapeptide FGVY and docking studies of the tetrapeptide was designed by using Schrodinger software. Qikprop (prediction of drug-like properties and %similarity with the existing drugs) results showed the ligand FGVY mostly act as antihypertensive and anticoagulant properties. The tetrapeptides\ was synthesized conveniently by solution phase technique. The synthesized compound was characterized by IR, ^{1}H NMR and FAB-Mass spectral studies. The synthesized peptide FGVY exhibited significant antioxidant activity.

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Table: 1 Docking of FGVY with HIF-1α (hypoxia-inducible factors) protein

Sl.no	FGVY Isomers	Docking score
1	D-Phe-Gly-D-Val-L-Tyr	-2.89
2	D-Phe-Gly-L-Val-L-Tyr	-2.45
3	L-Phe-Gly-L-Val-L-Tyr	-1.79

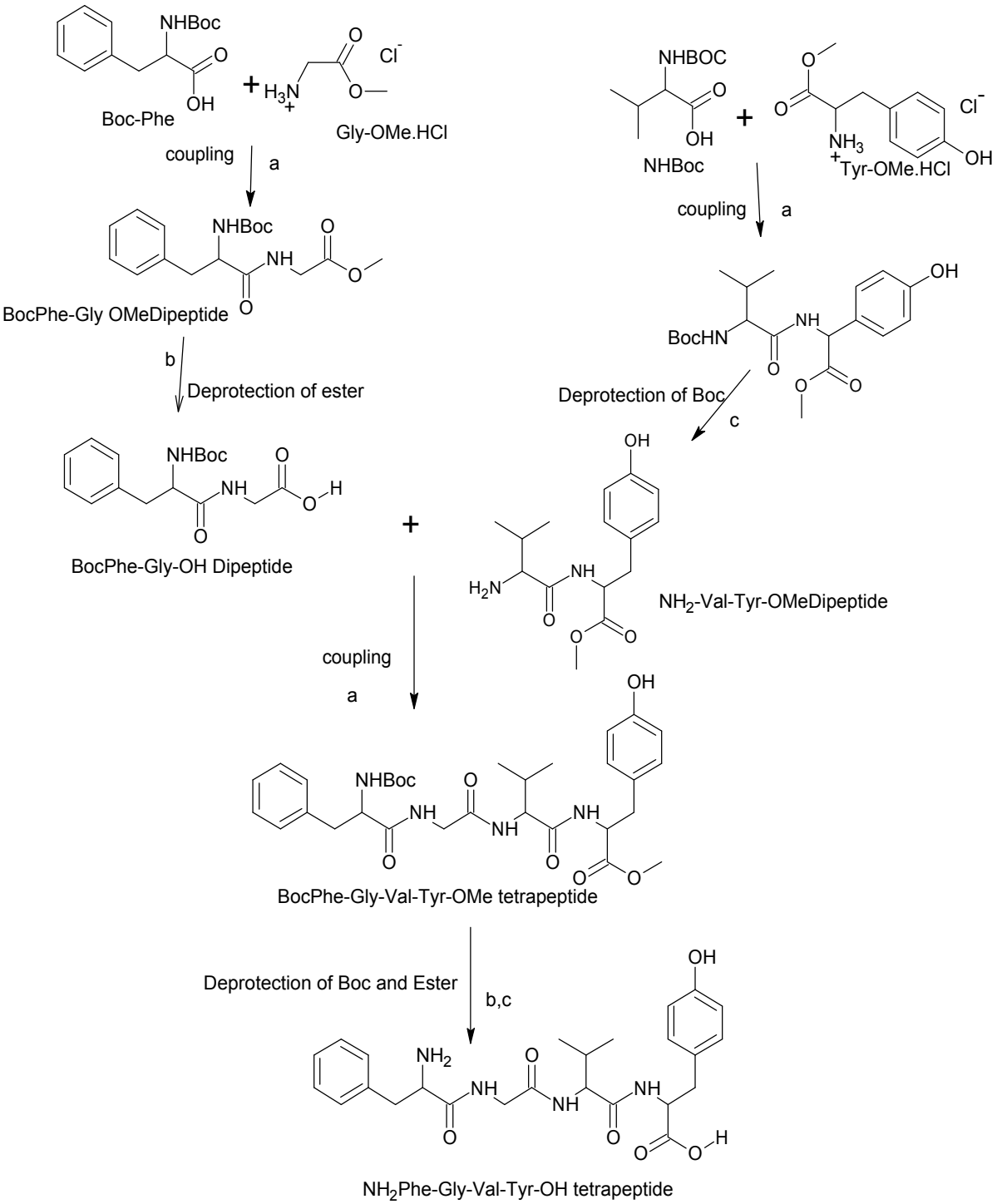
Table: 2 Physical data of synthesized peptide

Compound	Nature	% of Yield
D-Phe-Gly-D-Val-L-Tyr	Brown Semi solid mass	72

Table: 3 Antioxidant activity of synthesized peptide

Conc. (µg/ml)	Absorbance (Std)	% of inhibition (Std)	Absorbance (Sample FGVY)	% of inhibition (Sample FGVY)
10	0.1087	39.3076	0.16	11
20	0.0958	46.5103	0.13	33
50	0.0761	57.5097	0.10	44
100	0.0311	82.6353	0.09	50

Scheme 1



FGVY

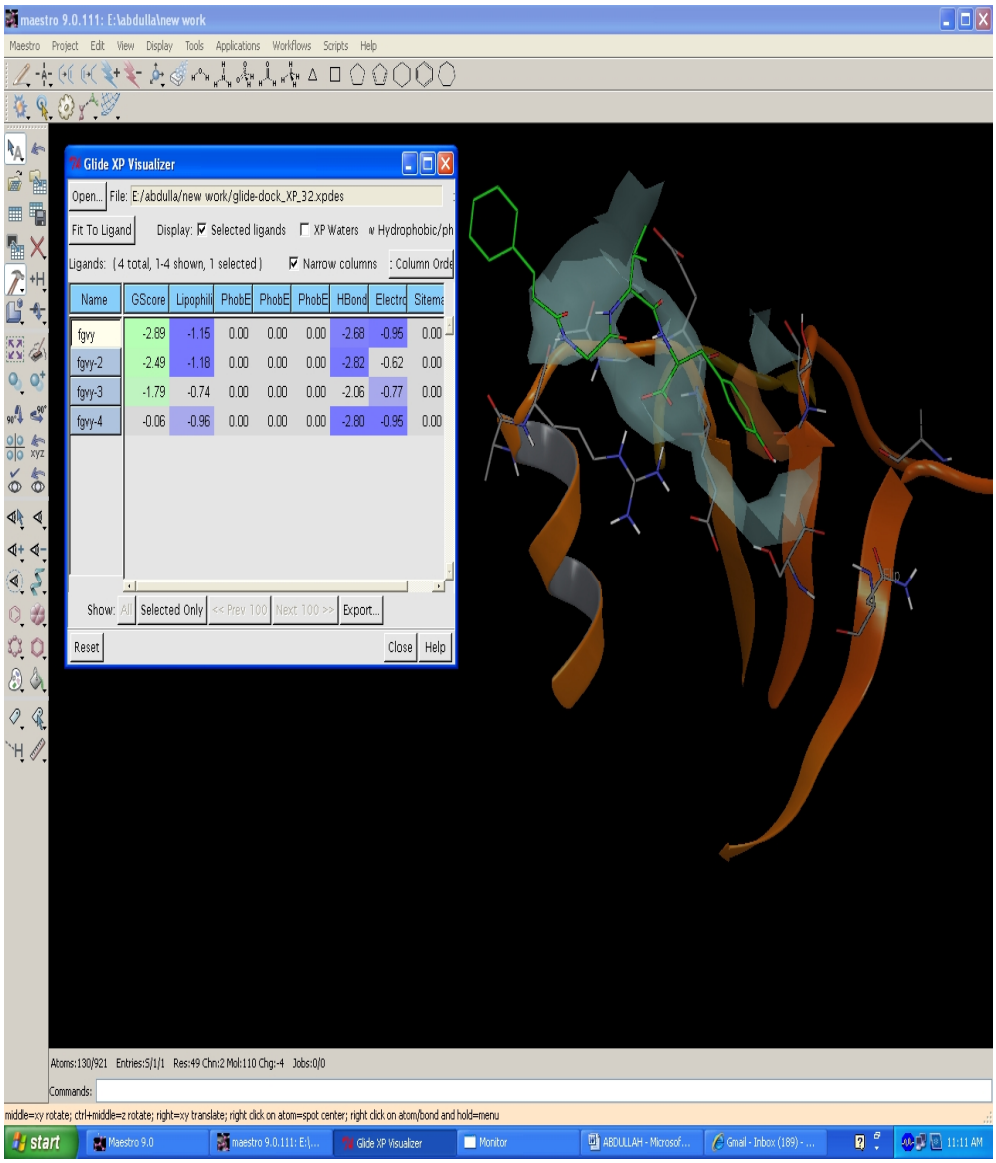


Fig-1: XP visualizer of docking of FGVY ligands with HIF-1α (hypoxia-inducible factors) protein.

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